

CLAIMS

1. A chemical conjugate between an immunoglobulin Fab fragment and molecular entities imparting diagnostic or therapeutic utility, whereby the only sites of conjugation on the Fab fragment are one or both of the sulphhydryl groups deriving from the selective and quantitative reduction of the inter-chain disulfide bond of said Fab fragment and whereby said molecular entities imparting diagnostic or therapeutic utility have at least one free sulphhydryl-reactive group, characterized in that the conjugation stoichiometric molar ratio molecular entity to Fab fragment is in the range from 0.95 to 1.05 or in the range from 1.95 to 2.05.
2. A conjugate according to claim 1, wherein a first of said sulphhydryl groups deriving from the selective and quantitative reduction of the inter-chain disulfide bond is quantitatively functionalized by reaction with one of said molecular entities imparting diagnostic or therapeutic utility.
3. A conjugate according to claim 2, wherein also the second one of said sulphhydryl groups deriving from the selective and quantitative reduction of the inter-chain disulfide bond is quantitatively functionalized by reaction with a second of said molecular entities imparting diagnostic or therapeutic utility, said second molecular entity being different or identical to the first one.
4. A conjugate according to claim 1, wherein both of said sulphhydryl groups deriving from the selective and quantitative reduction of the inter-

chain disulfide bond are quantitatively symmetrically functionalized by reaction with a stoichiometric excess of one of said molecular entities imparting diagnostic or therapeutic utility.

5. A conjugate according to claims 1 or 2, wherein one of said
5 sulphhydryl groups deriving from the selective and quantitative reduction of the inter-chain disulfide bond is chemically modified by reaction with a chemical moiety non-imparting diagnostic or therapeutic utility, said chemical moiety being preferably selected among protective groups of the thiol group or small alkylating or arylating agents.

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6. A conjugate according to claims 1 to 5, wherein said molecular entities imparting diagnostic or therapeutic utility comprise derivatives of chelating agents for, or chelates of, radionuclides, paramagnetic metal ions or luminescent metal ions, a chromophoric fluorescent or a phosphorescent
15 molecule, a biotin molecule, a hapten recognized by a distinct antibody or fragment thereof, an avidin or streptavidin molecule, a therapeutic drug, a lipophilic chain bearing molecular entity incorporated into liposomes, phospholipid-stabilized microbubbles, triglyceride- or polymer-based microspheres, microballoons which carry the diagnostic or therapeutic
20 agent.

7. A conjugate according to claim 6, wherein said molecular entities further comprise one or more functional groups which may be used, as such or after deprotection or after chemical modification, as targets for the
25 selective attachment of a second Fab fragment, equal or different from the first one, or of a second molecular entity imparting diagnostic or therapeutic utility.

8. A conjugate according to claims 1 to 6, wherein said sulphydryl reactive groups comprise iodoacetyl, bromoacetyl, vinyl, maleimido groups or polyfluorobenzene or dinitrofluorobenzene derivatives.

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9. A conjugate according to any one of the preceding claims, wherein the Fab fragment is a recombinant Fab.

10. A process for the preparation of the conjugates of claims 1 to 9,
10 comprising:

a) the selective and quantitative reduction of the inter-chain disulfide bond of a Fab fragment to give two free sulphydryl groups;

b) the quantitative functionalization of one or both of the sulphydryl groups from step a) with molecular entities having at least one free sulphydryl-reactive group and imparting diagnostic or therapeutic utility, to give mono- or diconjugate compounds, said diconjugates deriving from either symmetric or asymmetric functionalization of the sulphydryl groups.

20 11. The process of claim 10, wherein said selective and quantitative reduction of the inter-chain disulfide bond is performed with a phosphine, preferably tributylphosphine and tris-(carboxyethyl)-phosphine.

25 12. The process of claim 11, wherein the reducing agent is tris-(carboxyethyl)-phosphine.

13. The process of claim 11, wherein the reduction is carried out mixing

the reacting species under buffered conditions giving a final buffered aqueous reaction solution having the following characteristics:

Fab concentration : 1 – 100 μ M;
Phosphine concentration : 0.1 – 10 mM;
5 pH of the buffered solution : 4 – 8;
reaction time : 5 – 180 min;
reaction temperature : 4 – 45 °C.

14. The process of claim 13, wherein the preferred conditions are the
10 following:

Fab concentration : 1.5 – 10 μ M or 1 – 5 μ M;
Phosphine concentration : 0.5 – 5 mM;
pH of the buffered solution : 5 – 7;
reaction time : 25 – 70 min;
15 reaction temperature : 25 – 40 °C.

15. The process of claim 10, wherein said quantitative functionalization of step b) is performed immediately at the end of the reduction step a), in the same reaction medium, by adding a buffered aqueous solution of the
20 conjugating molecular entity, without purifying the reduced Fab fragment.

16. The process of claim 15, wherein the final buffered aqueous reaction solution has the following characteristics:

Fab concentration : 2 – 5 μ M;
25 Phosphine concentration : 0.5 – 5 mM;
conjugating moiety concentration : 0.1 – 100 mM;
pH of the buffered solution : 5 – 7;

reaction time : \geq 30 min;
reaction temperature : 4 – 45 °C or 20 – 40 °C.

17. *N²,N²-bis[2-[bis(carboxymethyl)amino]ethyl]-N⁶-[4-(2,5-dioxo-1*H*-pyrrol-1-yl)-1-oxobutyl]-L-lysine* as intermediate compound for the preparation of conjugates of claim 1.
18. Pharmaceutical compositions containing as active ingredients the conjugate compounds of claims from 1 to 9.
19. Compositions according to claim 18, wherein said conjugate compounds are formulated in the form of suspensions, solutions, emulsions for parenteral administration, lyophilizates to be reconstituted before use.
20. Diagnostic compositions according to claim 18, wherein the dose of the active ingredient ranges from 0.1 to 10 mg of conjugate per single administration.
21. Therapeutic compositions according to claim 18, wherein the dose of the active ingredient ranges from 10 to 500 mg of conjugate per single administration.
22. Compositions according to claim 18, for use in analytical immunochemical tests *in vitro*